

New Record of Western Flower Thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in South India

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Abstract— A necrosis disease of chrysanthemum caused by Tomato spotted wilt virus was reported in recent years in Tamil Nadu, a southern state of India. Whether *Frankliniella occidentalis*, the vector of TSWV is associated with the disease was looked into by analyzing thrips populations in these regions for morphometric and mtCOI gene sequences. The morphological key characters of the thrips populations studied fitted very well with the description given for *F. occidentalis*. A PCR amplified 358 bp fragment of mtCOI gene was analyzed for 24 individual thrips from 12 locations of Tamil Nadu. The consensus sequences revealed 92-100 % identity in the selected fragment with the *F. occidentalis* sequences in the database. Of the eight populations analyzed, two of them revealed 100 % identity with many sequences of *F. occidentalis*. However, one of the populations, FOTN6 was very distinct and exhibited only 92 % identity with all the *F. occidentalis* sequences compared. Totally there were 48 polymorphic sites out of 358 nucleotides compared, of which more than 65 % resulted in changes in the amino acids. The confirmation of presence of *F. occidentalis* in India is of paramount importance considering its role as active vector of tospoviruses present in the important ornamental high valued crops.

Keywords— *Chrysanthemum*, *Frankliniella occidentalis*, mtCOI gene, Tomato spotted wilt virus, vector.

I. INTRODUCTION

Thrips belonging to the order Thysanoptera are extremely small, rasping and sucking type of insects feeding on variety of cells and on various plant parts. They are polyphagous, infecting wide range of host species and are

more abundant in the tropics. Around 7,500 species of thrips have been recorded all over the world (Mound, 2009). Thrips cause economic loss on important crops by direct feeding. Indirectly they also cause significant damage through transmission of devastating viruses belonging to the genus *Tospovirus* of the family *Bunyaviridae*. There are fourteen species of thrips recognized as vectors (Riley *et al.* 2011).

Small size of thrips requires examination under a compound microscope at higher magnification. Such specimens must be specially prepared and placed temporarily or permanently on microscope slides (Schaeff, 2009). The probability of observing the full range of variability within any taxon depends on the size, number and dispersion of the available samples. The identification of specific thrips species on a host therefore becomes very difficult. The website describing definite morphological keys like <http://thripsnet.zoologie.uni-halle.de> (Cavalleri and Mound, 2012) is extremely reliable but difficult to apply for rapid identification due to small fragile nature of the insect.

In recent years, molecular markers are available which are extremely useful to resolve the species complex in many insect species (Frey and Frey, 1995). Molecular taxonomists have employed several markers like microsatellites, rRNA genes, ITS regions and Cytochrome b gene (Rokas *et al.*, 2002; Kjer, 2004; Kim and Sappington, 2005). Timm (2008) and Gloves *et al.* (2010) demonstrated the application of nucleotide sequence of mitochondrial cytochrome oxidase gene subunit I (mtCOI) in identification of thrips species. Brunner *et al.* (2002) analyzed the mtCOI gene of about 264 thrips representing 10 well defined species of thrips and found that the variation within species

was low, but divergence of about 18.6 % was observed between the species. Since mtCOI gene sequences have been found highly reliable and robust, Rebijith *et al.* (2012) extended the analysis of mtCOI gene for 151 species for the first time and found that intraspecific and intrageneric distance ranged from 0.0 to 7.9 % and 8.65 to 31.15 %, respectively. They also have established how mtCOI gene sequence analysis could serve as a barcoding system for identification of thrips species.

Correct identification of the insect up to species level is important from the plant quarantine view. It is necessary to identify the invasive exotic species of the insect without ambiguity in order to understand its entry point and the pathway of spread. Thrips are active vectors of viruses of which the diseases caused by virus species belonging to the genus *Tospovirus* belonging to the family *Bunyaviridae* are the most devastating and economically important. The active vectors of tospoviruses in India are *Thrips palmi*, *Thrips tabaci*, *Ceratothripoides claratris*, *Frankliniella schultzei* and *Scirtothrips dorsalis* (Singh and Krishna Reddy, 1996; Ravi *et al.*, 2006, Mandal *et al.*, 2012). It is not yet clear how many more thrips are involved in active transmission of tospoviruses in India. Recently, a necrosis disease caused by *Tomato spotted wilt virus* (TSWV) was recorded in chrysanthemum by Renukadevi *et al.* (2015) in Tamil Nadu, a southern state of India. The necrosis disease in chrysanthemum grown under protected cultivation was observed to show a spread of 0 to 6 % from the days the disease was recorded. This observation suggested slow spread of the virus by thrips. In order to identify the thrips prevalent in these locations, morphometric characters were critically examined. The mtCOI gene sequences were amplified from the thrips populations collected. On the basis of mtCOI sequence analysis and morphometric studies, presence of western flower thrips, the vector of TSWV was confirmed and the results are described in the present communication.

II. MATERIALS AND METHODS

Thrips specimens were collected from flowers of chrysanthemum, carnation, dahlia and gerbera at 12 locations of The Nilgiris and Salem districts of Tamil Nadu. The thrips were collected from the flowers by beating them over a white plastic tray. Thrips adhering to the plastic tray were picked up with a camel hair brush and preserved in 70 % ethanol in eppendorf tubes. The specimens were labeled properly and preserved at -80°C for molecular taxonomic studies. The details on location, crop and specimen IDs are given in Table.1. Specimens collected from a location were considered as one population.

2.1. Morphological identification

Thrips collected were transferred from the collection fluid into clean 70 % ethanol. The specimens were kept in 10 % potassium hydroxide for 2 days at room temperature for maceration and then transferred to absolute ethanol for 4 to 5 minutes for dehydration. A drop of Hoyer's medium was placed on the center of microscopic slide and the specimen was transferred to the slide. The wings, legs and antennae were spread by pressing on the basal segment with a fine needle and a cover slip was placed.

The slides were placed in hot air oven at 35 to 45°C for 4 to 5 days in a horizontal position for drying and a ring of shellac was applied on the edges of cover slip. Slides were labeled appropriately and preserved for taxonomy studies. Morphometric observations were recorded in five individuals from each population. The adult thrips specimens were observed under stereozoom microscope (LEICA MZ16) to capture the full view of adult thrips and the slides were observed and images were captured under phase contrast microscope (LEICA DM 750) at 10 x 40X for the key characters *viz.*, dark yellow body with brown areas medially on each tergite; 8-segmented antennae; antennal segments II and VI-VIII brown, III-V yellow with apices variably brown; III and IV antennal segment with forked sense cone; VIII antennal segment longer than VII; legs mainly yellow washed with brown; forewings pale with dark setae; head wider than long; 3 pairs of ocellar setae present, pair III longer than distance between external margins of hind ocelli, arising on anterior margins of ocellar triangle; postocular setae pair I present, pair IV longer than distance between hind ocelli; pronotum with 5 pairs of major setae; anteromarginal setae slightly shorter than anteroangulars, one pair of minor setae present medially between posteromarginal submedian setae; metanotum with 2 pairs of setae at anterior margin; forewing with 2 complete rows of veinal setae; tergites V-VIII with pair of lateral ctenidia, ctenidia sometimes weakly developed on IV, on VIII anterolateral of spiracle; posteromarginal comb on VIII complete, with short slender microtrichia arising from triangular bases; sternites III-VII without discal setae as described by Moritz *et al.* (2004).

Specimens collected from different locations were sent to Network Project on Insect Biosystematics, Division of Entomology, Indian Agricultural Research Institute, Pusa, New Delhi for species confirmation, registration and deposition in National Pusa collections. The specimens were also deposited in the Insect Biosystematics Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

2.2. Molecular identification

A single adult thrips was crushed in 10µl of sterile water in 1.5 ml eppendorf tube using a flame sterilized iron rod of 1.5 mm diameter and DNA was extracted following the procedure described by Asokan *et al.*, (2007). The crude extract was kept in boiling water bath for 5 min and followed by incubation at -20°C for 10 min. The extract was centrifuged for 5min at 4°C and 5 µl of the supernatant was used for PCR.

PCR was performed with the primers mtD7. 2F, 5' ATTAGGAGCHCCHGAYATAGCATT 3' and mtD 9.2 R, 5' CAGGCAAGATTAAATATAAACTTCTG 3' which targets 5' region of mtCOI gene (Brunner *et al.*, 2002). The primer was expected to yield an amplicon of approximately 500 bp length in different thrips species. Following were the PCR conditions; denaturing at 94°C for 30 sec, annealing at 53°C for 45 sec and extension at 72°C for 1 min. Amplification was done for 35 cycles and the final extension was for 20 min at 72°C. The reaction mixture for 25µl of total volume contained 5µl of template, 2µl of (15pmoles) forward and reverse primers and 12µl of master mix (Amplicon kit) and 4µl of water.

The amplicons were resolved on 1.2 % agarose gel containing ethidium bromide (10µg/ml) and electrophoresed along with molecular weight markers (100 bp and 1 kb ladder, Fermentas) at 80V in 1X TAE buffer. The gel was visualized in GELSTAN gel documentation system and images were captured.

The PCR products were eluted using GENELUTE gel extraction kit, Sigma and 150 ng of PCR products were sequenced in both orientations using the forward and reverse primers. Sequences were generated in an automated sequencer (ABI primer 310; Applied Biosystem, USA) by availing the commercial facility (Xcelris Labs Ltd.). Sequences were generated for two individuals of each population collected from 12 different locations.

The sequences were initially searched for homology in BLAST (www.ncbi.nlm.nih.gov) to confirm the thrips origin. The confirmed sequences were edited to equal length and compared with the sequences of thrips retrieved from database (www.ncbi.nlm.nih.gov). The identity between the sequences was compared by aligning the sequences in CLUSTALW alignment programme in Bioedit. Further, the phylogenetic relationship was analyzed using MEGA.6 software.

III. RESULTS

3.1. Morphological identification of *Frankliniella occidentalis*

From each population, five specimens were observed and they had following characteristic features. Adults are dark

yellow with brown areas medially on each tergite (Fig.1.a). Head wider than long. Antennae are eight segmented (Fig.1.b). VIII antennal segment is longer than VII segment. Antennal segments II, VI, VII and VIII are brownish; while, III, IV and V antennal segments are yellow with apices variably brown. Antennal segments III and IV are with forked sense cone (Fig.1.c). Pedicel of segment III is simple (Fig.1.d). Three pairs of ocellar setae present. Pair III is longer than the distance between external margins of hind ocelli, arising on anterior margins of ocellar triangle. Post ocular setae pair I present, pair IV longer than distance between hind ocelli (Fig.1.e).

Pronotum has five pairs of long setae. Apart from these setae, pronotum has four small setae between the major anteromarginal setae. Anteromarginal setae are slightly shorter than anteroangular setae (Fig.1.f). Metanotum has two pairs of setae at anterior margin. Forewings are pale with dark setae. Forewings have two complete rows of veinal setae (Fig.1.g). Legs are yellowish brown. Abdominal tergites V to VIII have pair of lateral ctenidia (Fig.1.h). Posteromarginal comb on VIII abdominal tergite is complete, with short slender microtrichia arising from triangular bases (Fig.1.i).

3.2. Molecular identification

In all the twelve population taken for the study, uniform amplicon of ~500bp was observed for all the samples. The quality of the product was good and the concentration of the amplicons was uniform (Fig.2). The amplicons of populations were sequenced in both the orientations. The length of sequence of the amplicon varied from 380-412 bp. Initially, homology search was performed in BLAST (www.ncbi.nlm.nih.gov). Of the twelve populations searched, hit was observed for eight populations FOTN1 to FOTN8. Maximum identity of 97-100 % was observed in this segment which corresponds to nucleotide co-ordinates 90 to 447 with many *F. occidentalis* entries, such as GenBank accession numbers GU148016, GU148084, GU148061, GU148020. The sequences of other four populations (FOTN9, FOTN10, FOTN11 and FOTN12) showed only 67-74 % identity with *F. occidentalis*. Since these sequences exhibit more than 18 % divergence, the sequences of these four samples were not considered for further analysis.

The composition of nucleotide revealed a high AT content (31.39 to 38.66 %). The sequences are deposited in GenBank with accession numbers KT907479 to KT907486. In order to study the relationship with other thrips species, the sequences of the population recorded in the present study and those of thrips species retrieved from NCBI database were edited to obtain uniform fragment length of 358 bp and aligned in CLUSTALW programme in the

BioEdit software. The alignment clearly revealed 48 polymorphic sites out of 358 nucleotides compared. The TN population of thrips showed 73-80 % identity with *Thrips palmi* (TP1-G1257207351, TP2-G1169238322), 74-81 % with *Thrips tabaci* (TT1-G1257207602, TT2-G1169238308) and 73-79 % with *Scirtothrips dorsalis* (SD1-FN546043, SD2-FN546042). On the basis of identity observed (Table 2) with twelve sequences of *F. occidentalis* derived from NCBI database,

F. occidentalis population from Tamil Nadu can be categorized into five groups. Groups 1 included, FOTN7 and FOTN8 populations which showed nearly 100 % identity with reference sequences. The 100 % identity was seen with GU148082, GU148084, GU148061, GU148020, AM932021, AM932017, KC008075 and HQ697596. The second group comprised FOTN1 and FOTN5 population which showed 100 % identity with two *F. occidentalis* sequences (GenBank Accession No. GU148082 and GU148016) and 97-99 % with other *F. occidentalis* sequences. The third group consisted of FOTN2 and FOTN3 population which shared 97-99 % with the reference sequences; the fourth group represented by FOTN4 population shared 95-96 % identity with all the referral sequences. The fifth group consisted of FOTN6 population which shared only 92 % identity. The most interesting feature is almost 65 % of the substitution resulted in the changes in the amino acid which is contrasting to earlier observation of Brunner *et al.*, 2010 and Rebijith *et al.*, 2012. It is especially true for the population FOTN 6 from Salem district which shared only 92 % identity in the nucleotide sequence. The changes in amino acids were at fifteen sites out of twenty three polymorphic sites. Some of the substitutions changes were common both for population FOTN4 and FOTN 6. The predicted amino acid sequences of the mtCOI amplified fragments of Tamil Nadu population were compared in multiple alignments with selected sequences of *F. occidentalis* (Fig. 3). From the graphic representation, it is clear that the population showed marked changes in amino acid profile.

The deviant nature of FOTN4 and FOTN6 population is very clear from the dendrogram drawn on the basis of multiple alignments of mtCOI gene nucleotide sequences. In the phylogenetic analysis, the species *Thrips palmi*, *T. tabaci*, *Scirtothrips dorsalis* were well separated from sequences of *F. occidentalis*. In the case of *F. occidentalis*, two distinct branches can be seen well supported by bootstrap value. The branch deviating had FOTN4, FOTN6 population along with *F. occidentalis* AM932025. The population FOTN1, FOTN2, FOTN3, FOTN5, FOTN7 and

FOTN8 grouped with other eleven *F. occidentalis* sequences showing 97-100 % identity (Fig. 4).

The individuals representing FOTN1 to FOTN8 populations have been registered at Network Project on Insect Biosystematics, Indian Agricultural Research Institute, New Delhi under the registration number RRS. No. 2117 THY, Code No.010-022.

IV. DISCUSSION

F. occidentalis is the most recognized invasive pest worldwide. Originally it was present only in the western half of North America from Mexico to Alaska (Tommasini and Maini, 1995). However, due to some genetic changes in the population, the outbreak of WFT was reported in Eastern USA in 1970s. From 1970s to 1990s it has spread across the continents like Europe, Africa and Australia (Kirk and Terry, 2003). In Asia, its presence has been recorded in Japan during 1990, in South Korea during 1994. In many of these countries the spread was clearly associated with the global trade involving horticultural crops. Wherever it has invaded, either it has spread in all the regions or confined to protected cultivations. In Costa Rica and Colombia, where chrysanthemum is grown in screen houses, it has not spread to other crops. Whereas, in Guatemala, its emergence was reported as field pest (EPPO, 2013). In India, until 2012, the presence of *F. occidentalis* has not been reported (Bhatti, 1980 and Rebijith *et al.*, 2012). Recently, Thyagi and Kumar, 2015 reported *F. occidentalis* in tomato from Bengaluru, Karnataka.

In the present study, the thrips species was confirmed as *F. occidentalis* on the basis of morphological characters. The morphological key characters observed were in accordance with the description of Moritz *et al.* (2004) and Tyagi and Kumar (2015). The species confirmation report from Network Project on Insect Biosystematics, Division of Entomology, Indian Agricultural Research Institute, Pusa, New Delhi also confirmed the specimens as *F. occidentalis*, the first report from Tamil Nadu and second report from India. In the present study, *F. occidentalis* was recorded in ornamental crops both in open and protected cultivation.

The main objective of the present study was to identify the thrips species prevalent in the Nilgiris and Salem districts of Tamil Nadu, where the incidence of necrosis disease caused by TSWV was recorded in chrysanthemum. Eight populations from eight different locations of the Nilgiris and Salem districts were characterized and identified both by morphological characters and through molecular taxonomy as *F. occidentalis*. Interestingly, of the eight populations, two were found to be distinct, showing more

than 65 % variation in amino acid residues.

The molecular markers are employed widely for precise identification of the insects like 16S rRNA, 18S rRNA, 28S rRNA, 5.8 rRNA and microsatellites. PCR combined with RFLP and ITS region has helped in differentiating the species and genera. Brunner *et al.* (2002) and Brunner and Frey (2010) demonstrated the application of mtCOI gene for differentiation of thrips species. They employed the universal primers of C1-J-1751 and C1- N- 2191 (Simon *et al.*, 1994) and observed that the size and quality of the amplicons varied among the species. On the basis of their analysis, they suggested the use of modified pair of primers mtD-7.2F and mtD-9.2R which was used in the present study. However, the amplicon was less than 500 bp length in the thrips population of the present study and the sequence generated were only 364 bp lacking the 5' 85-100 nt sequences obtained in all other thrips species and some *F. occidentalis* isolates. The regions aligned with nucleotide coordinate 90 to 447 with reference to *F. occidentalis* sequence of GenBank Acc. No. GU148082. The reduced amplicon size could be due to the difference in the primer binding region as observed by Asokan *et al.* (2007). Rebijith *et al.* (2012) used highly conserved LCO-1490 and HCO-2198 primers and observed that in no *F. occidentalis* isolate more than 500 bp length amplicon was obtained.

In the regions compared, nearly 100 % identity was observed in the population FOTN7 and FOTN8 with seven *F. occidentalis* sequences retrieved from GenBank. However, FOTN6 and FOTN4 were distinct. From the results of nucleotide analysis, it was clear that intraspecific variations are frequent. Analysis of sequence of *F. occidentalis* retrieved from data base also confirmed the above observations. The nucleotide changes (substitutions) in 48 positions resulted in the changes of amino acid residues too, at least in 65 % of them. This is contrasting to the observations of Rebijith *et al.*, 2012 and Brunner *et al.*, 2010 who has reported that the nucleotide substitution occurred only in the third position, not contributing to change in amino acid configuration. It is quite interesting to note that within 8 populations collected, deviation was observed in the population FOTN6 and FOTN4, which formed independent lineages.

The diverse nature of the population is more evident in phylogenetic analysis wherein the grouping of thrips into two main clusters was observed. In this, the population FOTN6 and FOTN4 branched separately. It is quite unexpected that within the distance of 200 km, the localities

from where thrips specimens were collected, population (FOTN6) showed variation. From the limited number of individuals analyzed and the area covered, it is not clear whether *F. occidentalis* in this area represents an introduced invasive population or existed here earlier. If *F. occidentalis* has been introduced in recent times, the population will be expected to exhibit uniformity. However, in the present study, minimum of five different population showing differences in amino acid residues were recorded. On the basis of which, it can be speculated that either variant population could have entered at different time points or could have evolved after entry at one time point and got adapted to new environment.

V. CONCLUSION

Our study has confirmed the presence of *F. occidentalis* in the Nilgiris and Salem districts of Tamil Nadu by molecular markers. Perhaps its presence was not detected earlier as the detection of specific thrips may depend on the crop plants and geographical locations surveyed. Whether the distribution is confined to flower crops or slowly expanding to agriculturally important crops is the next question awaiting answer. Since *F. occidentalis* is the active vector of tospoviruses especially TSWV, it is necessary that information on its occurrence and spread throughout India is generated. The present study is the second report of *F. occidentalis* from India. Whether *F. occidentalis* is wide spread in India and is contributing to the active spread of tospo viruses will have to be addressed to prevent the severe loss due to necrosis diseases in wide range of crops in future.

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Table.1: Collection of *Frankliniella occidentalis* specimens in Tamil Nadu, India

S. No.	Location Village/District	GIS Position	Crop	Sample ID	GenBank Acc. No
1.	Nedugula, Nilgiris dt.	11°47'N,76°89'E	Carnation (F)	FOTN1	KT907479
2.	Hubbathalai, Nilgiri dt.	11°34'N,76°76'E	Carnation (F)	FOTN2	KT907480
3.	Kokkode, Nilgiris dt.	11°43'N, 77°00'E	Gerbera (F)	FOTN3	KT907481
4.	Nandipuram, Nilgiri dt.	11°46'N,76°98'E	Gerbera (L)	FOTN4	KT907482
5.	Kodanadu, Nilgiris dt.	11°51'N, 76°90'E	Chrysanthemum (F)	FOTN5	KT907483
6.	Yercaud, Salem dt.	11°77'N,78°20'E	Chrysanthemum (F)	FOTN6	KT907484
7.	Hawakal, Nilgiris dt.	11°43'N, 76°90'E	Chrysanthemum (F)	FOTN7	KT907485
8.	Keirbetta, Nilgiris dt.	11°42'N, 76°87'E	Gerbera (F)	FOTN8	KT907486
9.	Hallimoyar, Nilgiris dt.	11°55'N, 76°94'E	Rose (F)	FOTN9	-
10.	Aracode, Nilgiris dt.	11°39'N, 76°90'E	Rose (F)	FOTN10	-
11.	Nanjanadu, Nilgiris dt	11°36'N, 76°60'E	Dahlia (F)	FOTN11	-
12.	Kethi, Nilgiris dt.	11°36'N,74°12'E	Dahlia (F)	FOTN12	-

F- Flowers; L-Leaves; dt.-District

Table.2: Thrips species used for nucleotide analysis

S. No.	Thrips species	ID*	GenBank Acc. No
1.	<i>Frankliniella occidentalis</i>	FO1	FN545981
2.	<i>Frankliniella occidentalis</i>	FO2	GU148084
3.	<i>Frankliniella occidentalis</i>	FO3	GU148061
4.	<i>Frankliniella occidentalis</i>	FO4	GU148020
5.	<i>Frankliniella occidentalis</i>	FO5	AM932025
6.	<i>Frankliniella occidentalis</i>	FO6	AM932022
7.	<i>Frankliniella occidentalis</i>	FO7	AM932021
8.	<i>Frankliniella occidentalis</i>	FO8	AM932017
9.	<i>Frankliniella occidentalis</i>	FO9	GU148082
10.	<i>Frankliniella occidentalis</i>	FO10	GU148016
11.	<i>Frankliniella occidentalis</i>	FO11	KCOO8075
12.	<i>Frankliniella occidentalis</i>	FO12	HQ697596
13.	<i>Thrips tabaci</i>	TT1	G1257207602
14.	<i>Thrips tabaci</i>	TT2	G1169238308
15.	<i>Thrips palmi</i>	TP1	G1257207351
16.	<i>Thrips palmi</i>	TP2	G1169238322
17.	<i>Scirtothrips dorsalis</i>	SD1	FN546043
18.	<i>Scirtothrips dorsalis</i>	SD2	FN546042

*Sequences retrieved from data-base are given these ids to facilitate presentation

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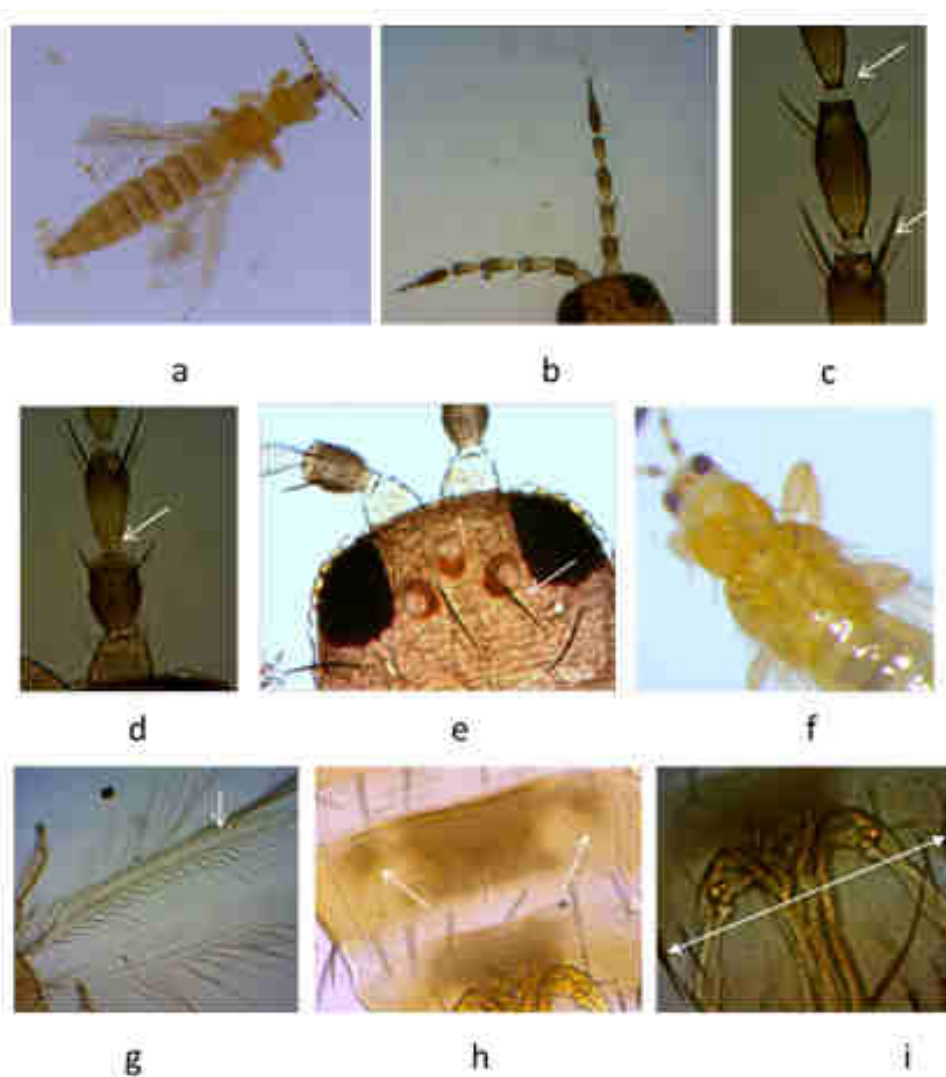


Fig.1: Morphometric key characters of *F. occidentalis*: (a) adult with medial dark yellow with brown areas on each tergite (stereozoom) (b) eight segmented antennae (c) antennal segments III and IV with forked sense cones (d) pedicel of antennal segment III simple (e) ocellar setae longer than distance between hind ocelli (f) pronotum with five pairs of long setae (stereozoom) (g) forewings with two complete rows of setae (h) abdominal tergites V to VIII with pair of lateral ctenidia (i) posteromarginal comb on VIII abdominal tergite is complete; all observations were recorded at 10x40x

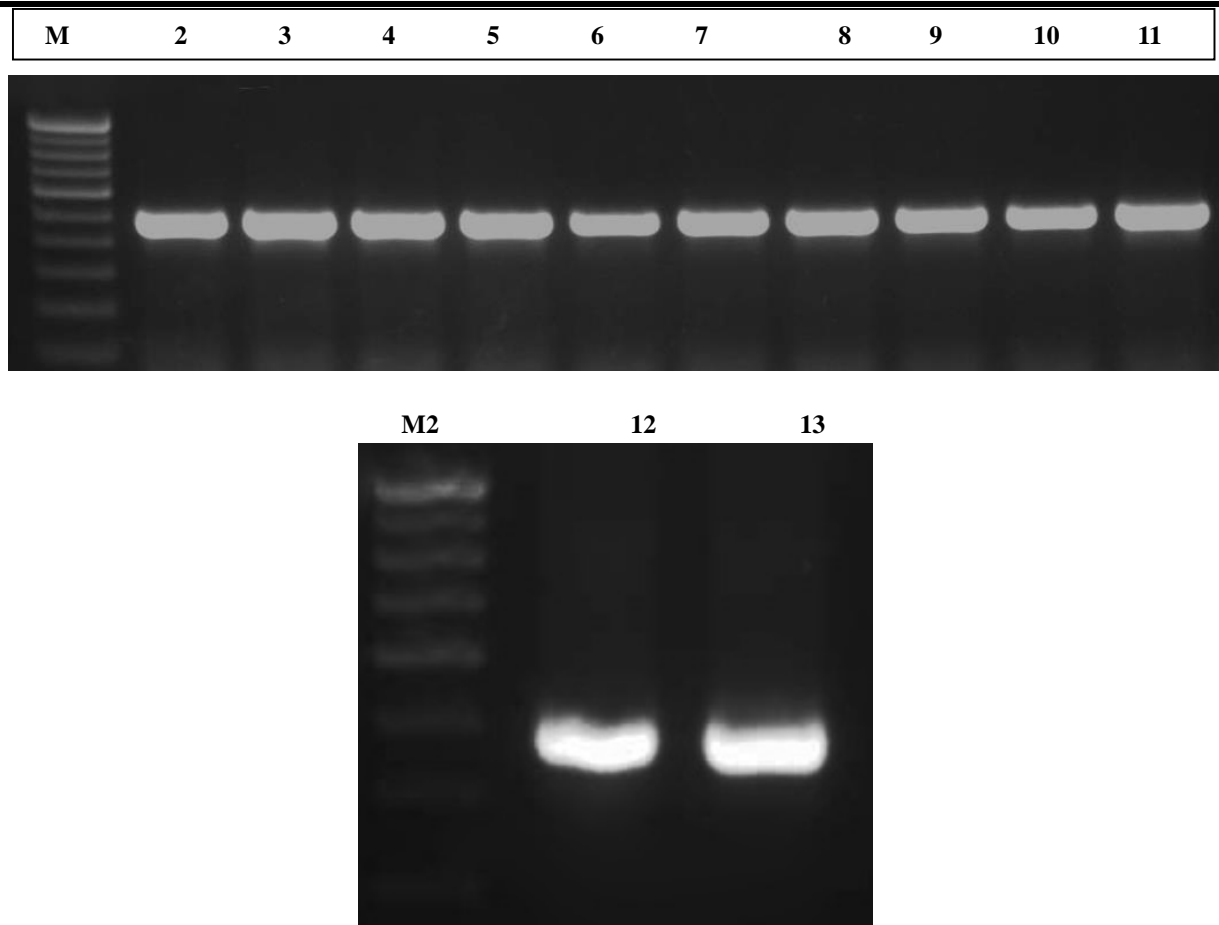


Fig.2: Agarose Gel electrophoresis of PCR amplicons of thrips population from Tamil Nadu: Amplification done with mtD7.2F and mtD 9.2R primers for mtCO1 gene. Lane 2 to 11: amplification of DNA extracted from FOTN1 to FOTN10 population. Lane 12 to 13: amplification of DNA extracted from FOTN12 and FOTN13 population. M1 - 100 bp ladder; M2 - 1 kb ladder.

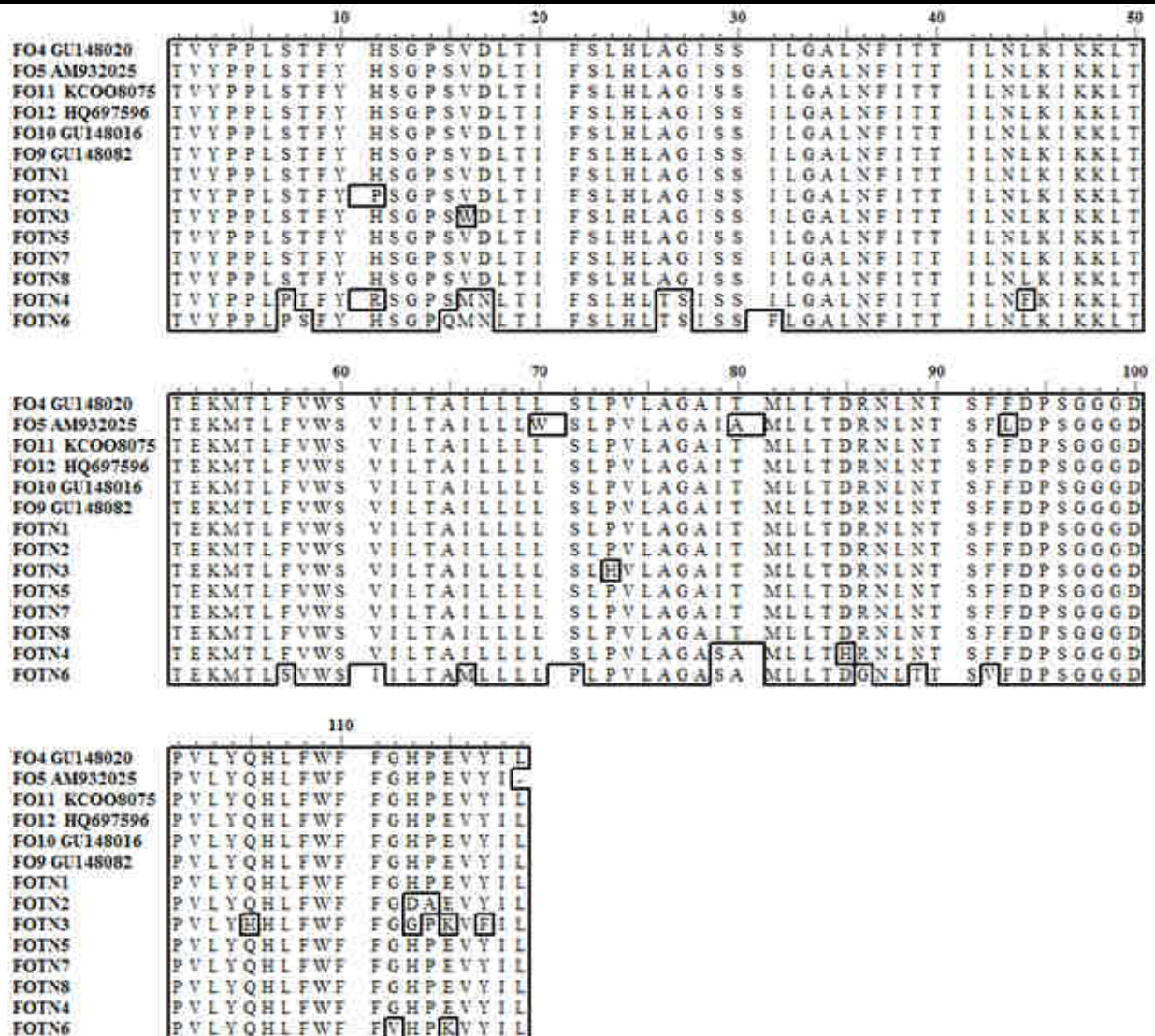


Fig.3: Multiple alignment of predicted amino acids sequences of mtCOI gene of *F. occidentalis* population from Tamil Nadu: The sequences of population FOTN1 to FOTN8 are compared with selected *F. occidentalis* sequences retrieved from NCBI data base. The identical sequences are boxed.

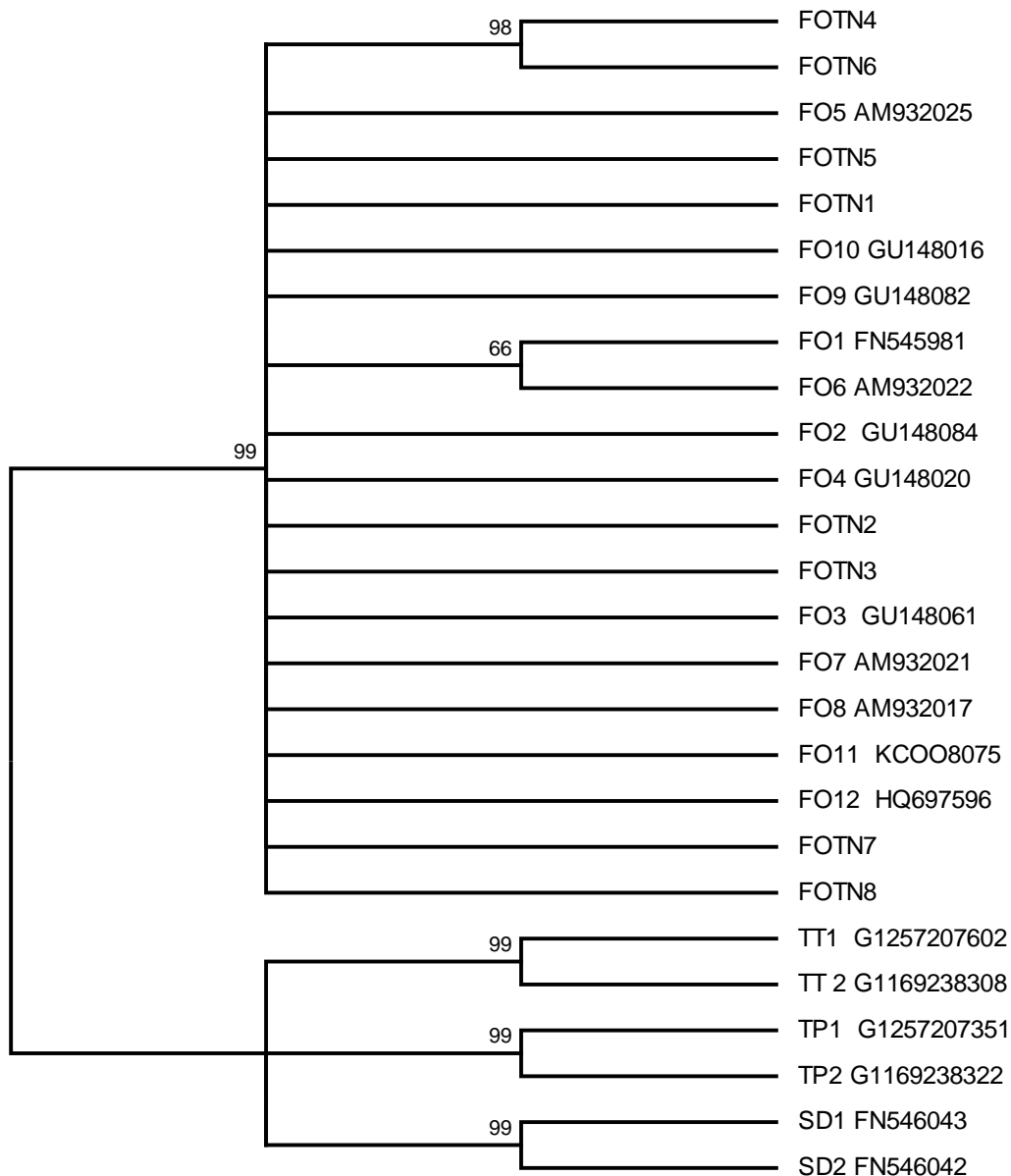


Fig.4: Phylogenetic tree constructed on the alignment of mtCOI gene fragment nucleotides sequence of *F. occidentalis* population: Tamil Nadu populations are labeled as FOTN. The sequences were aligned in MEGA 6 using CLUSTALW and the tree was constructed by neighbour-joining method. Horizontal distance is proportional to genetic distance amongst different thrips population. Vertical distances are arbitrary. The numbers at each node represent the percentage boot strap score (1000 replicates).